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Dear Mike:

I trust that you received those cultures OK which I sent you some time ago. These fermentative mutants are not the most stable things in the world, especially those involving glucose assimilation, and they really have to be repurified occasionally by streaking them out on EMB agar and repicking white colonies.

What's the latest on phosphorolysis in E. coli? I've gotten out a very active cell free prep. of b-galactosidase from K-12, using cells grown on lactose-synthetic medium and grinding them in the Booth Green Mill. Assays with O-nitropheyl galactoside which is working beautifully. The enzyme does not respond to phosphate, and is active in its absence (i.e. considerably less than 10^{-5} M), and I think must be taken as hydrolytic. Na^+ up to M/50 or higher stimulates about 100%, but is not essentially. Taking the "H enzyme" as the base, (i.e. low salt concentration), K and Cs are inert, as is NH_4; Li and especially Rb are inhibitory, the inhibition being reversed by either K or Na. A number of substituted NH_4 ions are also inhibitory. My conclusion is that the enzyme can function with varying efficiency with H^+, metal ions, etc., but that all of these compete with each other for the enzyme surface. Mg does not seem to be necessary; nevertheless, F^- is inhibitory only in the presence of phosphate, and the inhibition is slightly aggravated by the addition of Mg. Pyrophosphate is also inhibitory, but citrate is not. I wonder whether the fluoro-phosphate complex can tie up other non-Mg enzymes. On the other hand, Mg may be present, but with an exceedingly low dissociation constant. The enzyme preps can be purified to some extent with Ammonium sulfate pptn., and can be dried down with acetone without much inactivation. K_m with the chromogenic substrate is about 2×10^{-4}, with reasonably good linear $1/s$ — $1/v$ plots. Most reducing sugars tie up the enzyme, whether they are utilized by the organism or not, presumably competitively, but I'm still working out the details. The enzyme is formed in measurable amounts only after adaptation in lactose or other galactosides. It is not produced by any of the several different mutants tested. The one-to-one theory does not seem to apply here. For comparative purposes, I've been running some comparative tests on Snell's Lactobacillus bulgaricus. His growth observations are verified (i.e., lactose+, glucose+;galactose-). By grinding, I've gotten out a lactase which is much less active than the one from coli, is also stimulated by Na, but also needs Mg and probably phosphate, although myprepa aren't clean enough yet to be certain. The bulgaricus lactase is not nearly so rugged as the coli, and I'm having much more trouble with it.

Let's hear from you, best regards,